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RESEARCH ESTABLISHMENT  
LUCAS HEIGHTS**

**THE USE OF A RADIOACTIVE TRACER TO STUDY THE NESTING  
SYSTEM OF MASTOTERMES DARWINIENSIS FROGGAT**

by

**W. T. SPRAGG**

**R. E. FOX\***

**\* Forestry and Timber Bureau, Forest Research Institute, Darwin**

**January 1974**

**APPROVED FOR PUBLICATION**

**ISBN 0 642 99608 3**



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ABSTRACT

A new method for the tracing of termite nesting systems is proposed and proved in field trials with *Mastotermes darwiniensis* nesting systems. An attractive bait containing  $\gamma$ -ray emitting scandium-46 oxide is inserted into an infested site. The bait is eaten rapidly and taken to the nests where trophallactic transfer spreads the scandium through the colony members. The scandium is finally excreted and the excreta fixed in the gallery walls and carton of all sub-centres which can then be found using a  $\gamma$ -ray detector. The use of other radionuclides as colloids in similar bait is described.

This new method appears to introduce a new concept into the tracing of social insects.

*Note: This work has been submitted to a journal. Further details can be obtained from the authors or the Director of the Research Establishment.*

\* Forestry and Timber Bureau, Forest Research Institute, Darwin

National Library of Australia card number and ISBN 0 642 99608 3

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**BIOLOGICAL HALF-LIFE; COUNTING RATES; EXCRETION; GAMMA RADIATION; GOLD COMPOUNDS; INGESTION; INSECTS; RADIATION DOSES; RADIOCOLLOIDS; SCANDIUM OXIDES; SCANDIUM 46; TRACER TECHNIQUES; TRANSLOCATION**

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Figure 1 Field Trial I

Figure 2 Field Trial II



## 1. INTRODUCTION

The termite *Mastotermes darwiniensis* Froggatt has caused very considerable damage to experimental plantations of pines and introduced hardwoods in the Northern Territory of Australia. Successful methods of control have not been developed largely due to lack of information on the organisation of the colony, which does not build mounds or any other readily detectable structure. Since this termite is very primitive, it was suspected that its nesting system might be similar to that described by Grassi and Sandias (1893) for *Reticulitermes* with several nesting centres interconnected by a network of galleries.

Hill (1921, 1942) described nests of *M. darwiniensis* which he had found inside hollowed out trees, stumps, fence posts etc. Owing to the fragility of the subterranean galleries, he found it difficult and extremely time consuming to attempt to trace the nesting system by excavation but indicated that the foraging range could be of the order of 100 m. More recent attempts by Fox were equally fruitless. The present study was therefore directed to the development of a radioisotope method to trace the galleries and nests.

Conventional radioactive tracing techniques for insects depend on physical marking of the insect or feeding it some element as part of a metabolite which will remain in the insect. These methods have several disadvantages in tracing small insects underground. Physical marking permits a wide range of radionuclides to be used but requires that the insect be removed from its natural habitat to apply the marker. If sufficient radioactivity is used to enable detection underground, the radiation dose to the insect is very high. In the case of termites, owing to the soft cuticular surface, any physical mark is limited to the head parts, nearest to the cervical nerve ganglia which would receive the largest radiation dose. Using a metabolite, the choice of radioisotope which can be fed to an insect is severely limited; the nuclear properties of any suitable radioisotope are unsuitable for the work while the amount ingested would be small. In fact, for these investigations, it is not necessary to know the movement of any one termite and the ideal method would be to make the termites mark out their own trails.

Such a method would consist of permitting the termites to mark themselves by ingestion of food containing a physiologically inactive radiotracer which would pass through the insect within a few days and which, during that time, would follow the normal routes of trophallactic transfer to other castes thus rapidly reducing the radiation dose to any one insect. The radioisotope would be excreted by all the colony members and, as the excreta are incorporated in the gallery walls and carton of the nest, the nest would become radioactive. This possibility poses three separate problems, (i) to select a non-toxic radioisotope in a chemical form having a short biological half life, a longer physical half life, and emitting energetic  $\gamma$ -radiation, (ii) to provide a food which would be preferentially consumed by the termites in the presence of ample natural food and (iii) to ensure that the radioisotope would pass through the insect to other members of the colony by the normal methods of trophallaxis being finally excreted by all castes. It was presumed that the excreta would be disposed of in the gallery walls and carton of the nests.

## 2. LABORATORY TRIALS

Scandium-46 ( $t_{1/2}$  84 days,  $\beta_{\max}$  0.39 MeV and  $E_{\gamma}$  0.89 and 1.12 MeV) seemed very suitable as its biological half life in mammals has been reported as approximately 24 hours and it seemed likely that this would also be true for insects. The large  $\gamma$  yield would facilitate detection while the comparatively low  $\beta$  energy would minimise radiation dose to the insect. Preliminary trials using cellulose suspended in agar gel showed that the insects would not touch baits containing scandium chloride in solution but accepted baits containing finely ground scandium oxide in suspension.

Laboratory trials were made on groups of 25 insects in petri dishes provided with filter paper impregnated with the suspension of scandium oxide in agar. These showed that the scandium was ingested with cellulose, that trophallactic transfer occurred to soldier castes and to other workers and that the excreta contained the scandium. The biological half life was found to

be approximately 24–36 hours. Considerable variability in uptake was observed. With high uptake, reasonably consistent results were obtained but with low uptake excretion rates were significantly less. The biological half life of the scandium in soldier castes was about 5 days.

Experiments were undertaken to find a more attractive bait. Although cellulose was satisfactory in initial laboratory trials, uptake was very irregular and obviously unsuitable for field work. Better uptake was obtained from baits made from sawdust in agar gel but it was observed that the larger particles of sawdust were being rejected. Using sawdust of particle size  $< 500 \mu\text{m}$ , uptake was very much improved. The addition of other nutrients such as glucose, trace amounts of calcium, nitrates etc. had no effect. Sawdust made from *E. regnans* was used for the first field trial. However, subsequent free choice experiments showed that *E. tetrodonta* (Northern stringybark) was the most effective of the woods tried.

The final field baits consisted of a paste of the sawdust in 1% agar containing  $^{46}\text{Sc}$  as finely ground oxide of specific activity  $1 \text{ mCi mg}^{-1}$ . The material was packed into a 1 cm diam. hole drilled up the centre of 1.25 cm diam. commercial dowelling. About 4 g of this paste could be packed in a 10 cm deep drill hole. The dowelling could then be inserted into a 1.3 cm diam. hole drilled in an infested tree.

### 3. RADIATION DOSE TO INSECTS

A rough calculation can be made of the magnitude of the radiation dose received by one termite. Assuming that it weighs 50 mg, is 1.1 cm long and ingests  $1 \mu\text{Ci } ^{46}\text{Sc}$  in 5 mg bait, the  $\beta$  dose would be of the order of  $6 \text{ rads hr}^{-1}$  in the absence of other termites. As transfer of food is rapid the total dose received would not exceed 20 rads. A similar calculation of the  $\gamma$  dose within the nest volume assumed to be 10 cm diam., shows that  $1 \text{ mCi } ^{46}\text{Sc}$  distributed throughout the nest would give a  $\gamma$  dose of  $0.4 \text{ rads hr}^{-1}$ . As no more than  $20 \mu\text{Ci}$  are required for detection of a nest in a tree the residual  $\gamma$  dose can be neglected.

### 4. FIELD TRIAL I

In an uncleared block of open eucalypt forest in the Howard Springs reserve approximately 32 km from Darwin, infested trees were found using the Greaves and Florence technique (1966). Previous attempts had been made in this area to excavate *M. darwiniensis* nesting systems. From a group of trees some distance from the nearest excavation an *E. tetrodonta* was selected as the bait tree. Two dowels each containing  $200 \mu\text{Ci } ^{46}\text{Sc}$  were inserted into the tree and uptake of the bait observed over two days. About 75% of the bait was removed and radioactivity was observed in nearby trees using a portable scintillation detector. At this stage no further bait removal occurred. At weekly intervals four further baits were inserted into nearby trees which had already become radioactive. Baiting was stopped when no further spread of activity was observed. Figure 1 shows the sites of detection of  $^{46}\text{Sc}$  and other sites where *M. darwiniensis* was found but no radioactivity. A total amount of  $1 \text{ mCi } ^{46}\text{Sc}$  had been distributed among 31 sites within an area of 80 by 84 m. Trees 2, 3 and 4 may have been within the experimental area. They had been much disturbed during the earlier excavation attempts and may have been vacated. Bait consumption was satisfactory but not as vigorous as hoped. However, 4 hours after introduction of the bait into the second tree, increased levels of radioactivity were detected in three trees 5, 6 and 13 m from the bait tree.

A shrub, 2 logs, a stump and 3 trees where deposition had occurred were cut open and examined. Sub-centres were present in all cases, the  $^{46}\text{Sc}$  being found fixed in their structural material. The sub-centres were similar to those described by Hill (1942). In one of the felled trees, a sub-centre was found 26 m above ground level, indicating that search should have been carried out to much greater heights than the 2 m which had been the limiting height used. Samples of termites were taken from sites throughout the area after the experiment and counted in a well counter scintillation detector in groups of 5. Out of a total of 600 insects no radioactivity was found. This first experiment proved the value of the new method for finding nests. However, a search for indications of interconnecting galleries was completely unsuccessful.

## 5. FIELD TRIAL II

As no gallery system had been detected in the first trial a repeat using larger amounts of  $^{45}\text{Sc}$  was carried out in an area within the Howard Springs reserve which had not previously been disturbed. As a result of the free choice experiments *E. tetrodonta* sawdust was used instead of the *E. regnans*. In this case four bait dowels, each containing 1 mCi  $^{45}\text{Sc}$  were inserted one at a time at 2 day intervals into one tree. The baits were removed rapidly and completely by the termites and 36 radioactive sites were found in an area of 60 x 54 m. Although infested with *M. darwiniensis* no radioactivity was found in trees outside this area. The results are shown in Figure 2. All sites where radioactivity was found were drilled and blank dowels inserted immediately baiting was stopped. The presence of *M. darwiniensis* was confirmed at every site. Two weeks after the experiment the blank dowels were examined again. Two trees on the edge of the marked area were found to have been invaded by *Coptotermes acinaciformis* Froggatt. The site has been left undisturbed for future studies on change of colony distribution.

A search for galleries within the system was again completely unsuccessful. On this occasion both a scintillation type counter and a geiger counter assembly in the form of three 20 cm G.M. tubes in line were used.

It was considered that the radioactivity associated with the sub-centres would probably decrease exponentially with distance from the bait tree. Radioactivity measurements were therefore made at a fixed distance from the centre of each marked sub-centre. While the results were only approximate due to variation in attenuation by the wood of the trees, the variation in radiation levels was completely random (Table 1).

TABLE 1

### RADIOACTIVITY OF SUB-CENTRES - FIELD TRIAL II

A = Distance from bait tree in metres

B = Counts  $\text{sec}^{-1}$  - background

A	B	A	B	A	B
3	1400	18	133	27	864
3	532	18	80	27	9700
13	67	21	227	28	200
14	67	23	100	29	69
14	667	24	93	29	260
17	532	24	100	29	532
17	3200	24	167	29	2667
17	100	25	160	31	67
17	260	27	67	33	100
18	386	27	67	35	67
18	220	27	80	38	80

Bait tree 8400 counts  $\text{sec}^{-1}$

While the bait tree in trial II showed 8,400 counts per second (cps) 2 trees at 3 m distance showed 1,400 cps and 532 cps. A tree at 29 m distance measured 2667 cps and another at 27 m distance 9,700 cps. An approximate calculation of the total radioactivity associated with the active sites indicated that almost all of the activity taken from the original baits could be accounted for in the nests.

As in the previous trial, baiting was continued until no further spread of active sites was found. To establish that this technique adequately defines the colony boundary, further baits should be used in the boundary area. This has not yet been done.

The technique was again used successfully at the Thorac Agricultural Reserve near Darwin to obtain large samples of *M. darwiniensis* for laboratory studies. The speed and rate of spread was similar to that seen in the second trial.

## 6. RADIOACTIVE COLLOIDS

The long half life of  $^{45}\text{Sc}$  prevents studies on colony change over short periods, while the particle size distribution of the scandium oxide is difficult to control during preparation. It was therefore considered that a more satisfactory product would be obtained by using a colloid prepared in the agar solution. Radioactive colloidal gold was chosen for the first attempt to provide a wider choice of radionuclide and improved particle size distribution. Radioactive gold foil of specific activity  $10 \text{ mCi mg}^{-1}$  was dissolved in aqua regia and the acid removed by careful repeated evaporation to near dryness. The gold chloride was dissolved in hot 1% agar solution and reduced by the addition of a trace of ascorbic acid. The resulting reddish purple sol was used to make up a paste with the *E. tetrodonta* sawdust.

Laboratory trials using this preparation on *M. darwiniensis* showed much more regular uptake by workers and similar trophallactic transfer to soldier and other workers. The biological half life is about 24 hours and the gold is finally excreted by all castes.

## 7. DISCUSSION

The method described appears to introduce a new concept into the tracing of social insects. There are several advantages (i) it is not necessary to disturb the insects in their natural environment, (ii) a wide range of radionuclides can be made available by choosing a compound insoluble in body fluids, in the form of finely ground particles or in colloidal form and (iii) the radiation dose to the individual insect is negligible. Possibly the most important feature is the design of a bait which seems to be uniquely attractive to the foraging caste. The method need not be restricted to solid baits as colloids can be readily added to sugar solutions or honey. As the possible colloids which could be used in liquid baits would mostly be hydrophobic it would be necessary to add some protective agent such as gelatin or agar in low concentration to maintain stability.

While the study is in a very early stage, the results are quite remarkable. The variation in count rate among the detected sites was from 60 cps to 9,700 cps. Of the three most active sites found in trial II with count rates of 9,700, 3,200 and 2,667 cps, their distances from the bait tree were 27, 17 and 29 m respectively, while the only two sites within 3 m showed count rates of 1,400 and 532 cps. The total count rate, as measured from a fixed distance from each site was 33,000 cps and therefore 30% ( $9,700/33,000$ ) of the bait, i.e. 6 g, was consumed and moved 27 m from the bait tree. If we estimate that each termite ate the equivalent of 10% (5 mg) of its body weight, then 1,200 termites took part in this 'long haul'. The simplest explanation is that the levels of marking are measures of centre size. The fact that the termites should forage through 27 m of ample natural food to eat a 4 g bait would appear to be a measure of attractiveness of the bait but this would also assume an active and rapid communication system throughout the whole colony. Alternatively, the most radioactive sites might be sites of maximum building activity and that an 'immediate demand' situation arises during any period of rapid nursery construction.

The nests are primitive and may well be built and abandoned at frequent intervals. To check such hypotheses it would be necessary to label the nests at frequent intervals and this cannot be done using the comparatively long lived  $^{45}\text{Sc}$ . The use of colloidal gold containing  $^{199}\text{Au}$  makes it possible to carry out such observations at 3 week intervals. The rapidity of bait uptake and excretion is so rapid that even shorter lived radionuclides could be used.

#### 8. REFERENCES

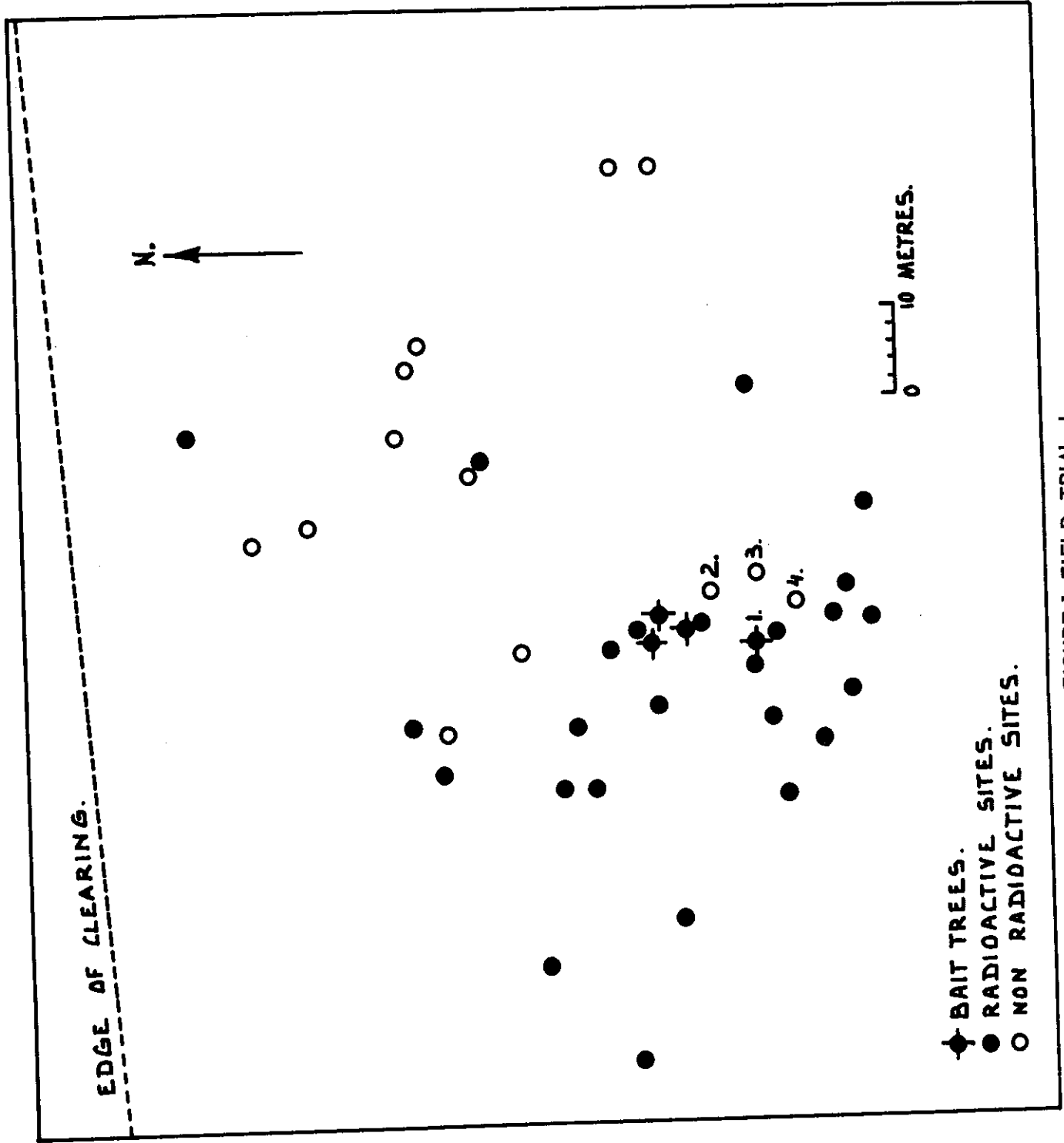
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- ◆ BAIT TREES.
- RADIOACTIVE SITES.
- NON RADIOACTIVE SITES.

FIGURE 1 FIELD TRIAL I

- LEGEND**
- ◆ Bait Tree
  - Radioactive Site - living tree
  - " " - dead tree
  - " " - log or stick
  - Other living trees
  - Non-radioactive masto site
  - 30 + cm
  - 20 to 30 cm
  - 10 to 20 cm
  - 0 to 10 cm

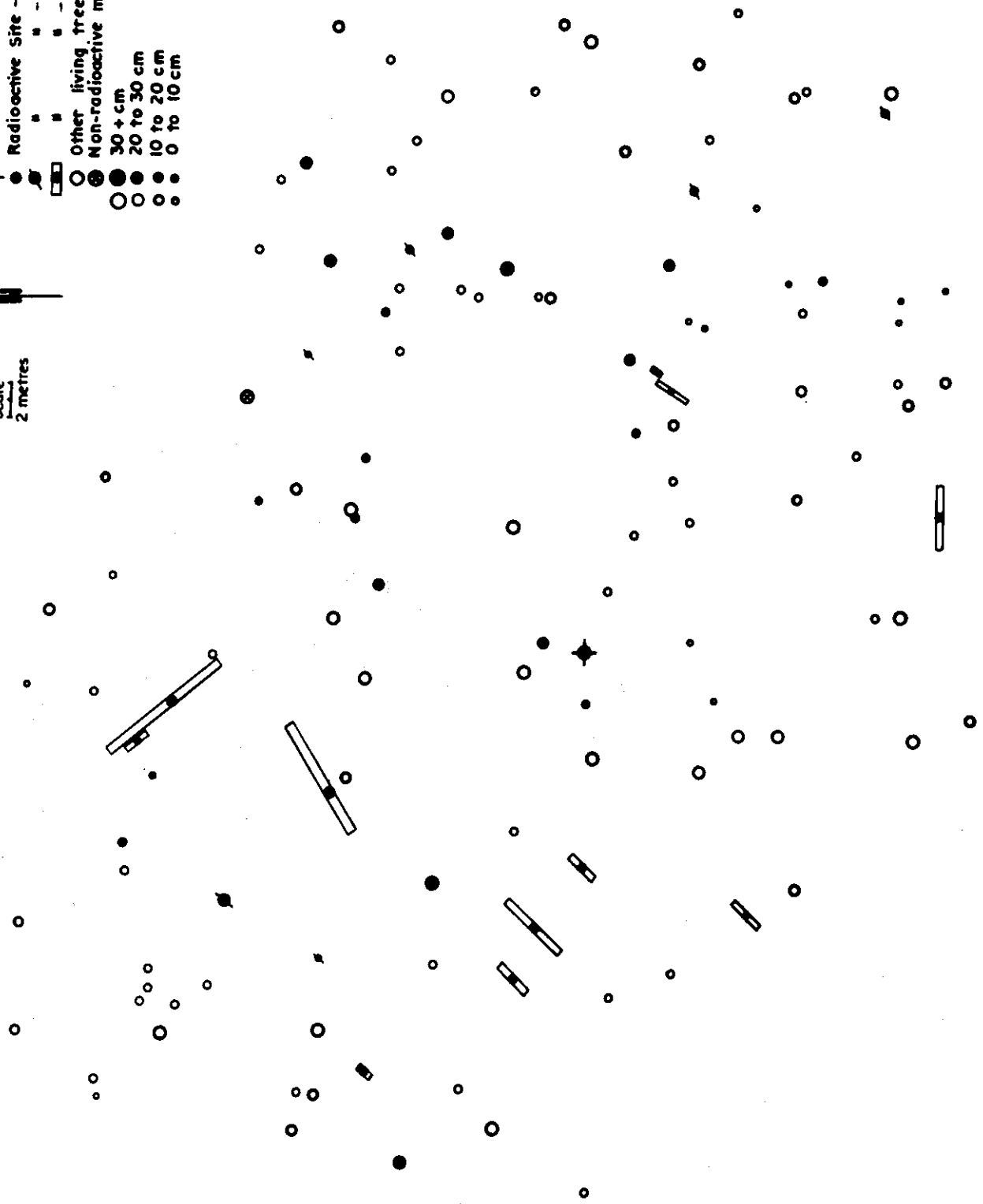
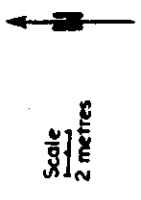


FIGURE 2 FIELD TRIAL II